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A SUMMARY OF THE NATIONAL INSTITUTES OF HEALTH (USA) GUIDELINES FOR RECOMBINANT DNA RESEARCH

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On June 23, 1976, Donald S. Fredrickson, Director, National Institutes of Health, announced publication of guidelines[1] designed to eliminate or minimize any potentially hazardous consequences of what has been called recombinant DNA research. The guidelines were subsequently published in the United States Government publication, the Federal Register (Part II for 7 July 1976).

The promulgated guidelines are the result of a year and a half of intensive work by the NIH Recombinant DNA Molecule Program Advisory Committee*

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(hereafter called the Advisory Committee) as well as consideration of a variety of views expressed to the Director either in writing or at a public hearing in February 1976. A summary of the history of the development of the guidelines as well as the various views expressed by many commentators is given in the Director's Decision Statement[1], which accompanied publication of the guidelines.

For the purposes of the guidelines recombinant DNA experiments are defined as those involving molecules that consist of different segments of DNA which have been joined together in cell-free systems, and which have the capacity to infect and replicate in some host cell, either autonomously or as an integrated part of the host's genome. Fig.1 depicts a generalized recombinant DNA experiment and defines certain terms as they are used in the guidelines.

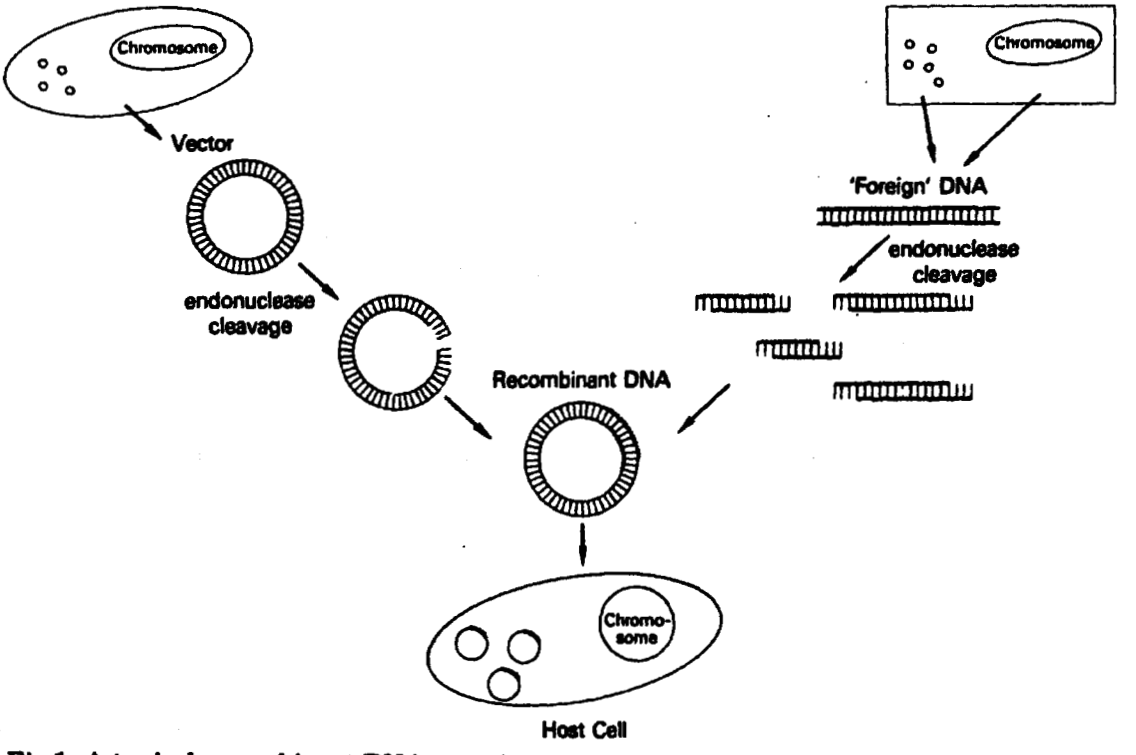

Fig.1. A typical recombinant DNA experiment.

At the upper left is a cell containing chromosomal DNA and several small independent genetic elements. These small independent DNA molecules are isolated from the cell and serve as one portion of the recombined DNA, the segment termed the vector. Such elements may be circular DNA molecules such as plasmids, or viral DNAs, and they can be cleaved, as shown, by restriction endonuclease (or by other means) to yield linear duplex DNA strands with either sticky ends, or ends that can be made sticky by appropriate modification. In most experiments, the recombinant DNA will finally be reinserted into cells of the same species from which the vector was isolated.

It is the genetic information encoded in the DNA of the vector which ultimately will be responsible for the continued existence and replication of the recombined DNA in the recipient cell. At the upper right of Fig.1 another cell is shown as a rectangle. This cell will serve as the source of DNA to be joined to the vector. This DNA is termed the foreign DNA, and the rectangular cell can represent a cell from any living species. As shown here, the foreign DNA might contain chromosomal DNA or independent DNA elements, or both. It too can be cleaved to yield fragments of various lengths with sticky ends, or ends that can be made sticky. The foreign DNA fragments are then joined to the vector DNA, usually by duplex formation at the sticky ends, followed by closure of the internucleotide bonds with polynucleotide ligase. The recombinant DNA is subsequently inserted into a recipient cell, which is called the host. Again, the host cell will most likely be of the same species as that used for the isolation of the vector. The cells are then placed under conditions where either they or the recombined DNA can replicate.

In the experiments discussed in the guidelines the host cells are generally single living cells, either microorganisms such as bacteria, or animal or plant cells grown as single cells in tissue culture.

General principles. The guidelines start with a statement of general principles and these are consistent with the general conclusions published in the report of the International Conference on Recombinant DNA at Asilomar, California, in February of 1975[2]. (*1*) The first principle is that there are certain experiments which, in the light of currently available information, may be judged to present potential hazards of so serious a nature, that they should not be attempted at this time. (*2*) A large group of feasible experiments appear to pose lesser or no potential hazard, and can therefore be performed provided that the information to be obtained, or the practical benefits anticipated, cannot be obtained by conventional methods, and provided that appropriate safeguards for containment of potentially hazardous organisms are incorporated into the design and execution of the experiment. (*3*) The more serious the nature of any possible hazardous event, the more stringent should be the safeguards against escape of the potentially hazardous agents. The safeguards should be at least as stringent as those generally used to handle the most hazardous parent of the recombinant. Since the potential hazards and their estimation are conjectural and speculative, the levels of containment required for potentially hazardous organisms should be set high initially, and modified only when there is substantial relevant information to advise such modifications. (*4*) The guidelines are to be reviewed at least annually in order to account for new information.

Containment methods. Three approaches to the problem of containing potentially hazardous organisms form the basis of the safeguards recommended by the guidelines. Each of the three may be viewed as setting up barriers to the dissemination of potentially hazardous organisms from the laboratory situation, and as setting up barriers between the laboratory worker and the

organisms. Two of these approaches involve the limitation of the actual physical escape of the organisms, and are referred to as *physical* containment. The *first* such approach is the set of *standard microbiological practices*, that have been developed over a period of many years, and are widely used for handling pathogenic organisms both in research and clinical laboratories. In the hands of well trained personnel, these procedures have proven to be effective in safeguarding both the worker and the environment from the spread of pathogenic agents. The *second* approach to physical containment involves the use of *special kinds of equipment and facilities* (*1*) to limit spread of aerosols, (*2*) for decontamination and containment of laboratory air and wastes, and (*3*) limitation of access to laboratories. As with the standard microbiological techniques the type of equipment and facilities are not new, but have been developed and used previously for containment of known pathogenic organisms.

The guidelines go into some detail concerning the practices and facilities required for physical containment: four levels of physical containment are specified. They are termed P1, P2, P3 and P4 in the document, in the order of increasing levels of containment. P1, the lowest level, consists of the use of the standard microbiological practices mentioned before. The P2 and the next higher level P3, each require special procedures and facilities (including vertical laminar flow biological safety cabinets and laboratories maintained at lower air pressure than the surrounding building) designed to limit to increasing extents any possible accidental escape of potentially hazardous organisms. Finally, P4, the maximum level of containment requires sophisticated and isolated facilities designed for maximum containment. Each of the levels, P2 through P4, assumes that the techniques demanded by P1, the standard microbiological practices, will be followed. Furthermore, for each level, *relevant training* of personnel is mandatory. The training is to include the nature of the potential hazards, the technical manipulations, and instruction in the biology of the relevant organisms and systems. Specific emergency plans, to be used in case of accident, are required and serological monitoring, where appropriate, is to be provided.

The *third* approach to the problem of containing potentially hazardous organisms within the laboratory is the use of biological barriers. *Biological containment* is defined as the use of host cells and vectors with limited ability to survive outside of very special and fastidious laboratory conditions that are unlikely to be encountered by escaped organisms in natural environments. Biological containment is an integral part of the experimental design, since the host and vector will need to be chosen, in any given experiment, with a view both to the purpose of the experiment and to containment. The guidelines stress that physical and biologic containment procedures are complementary to one another each one serving to control any possible failure in the other. The use of both in a given experiment affords much higher levels of containment than either one alone. Therefore, the guidelines always recommend both a particular level of physical containment, and a level of bio-

logical containment for any given experiment. The guidelines explicitly recognize that novel techniques which enhance physical and biological containment capabilities are likely to be evolved as research proceeds and may reduce the needs for the standard physical containment procedures. Such innovations are to be considered as part of the on-going review of the guidelines for appropriate revision.

Publication. The guidelines recommend that publications describing work on recombinant DNA include a description of the containment procedures used.

Experiments to be deferred. The first class of experiments described in the guidelines are those which are not to be carried out at the present time. While it may be argued that a combination of P4 physical containment and a high level of biological containment could essentially contain these recombinants, the magnitude of the possible dangers, were containment to fail, dictates that these experiments be deferred. This class of experiments includes the following.

(*1*) Any experiments in which a portion of the recombinant DNA derives from pathogenic organisms listed under classes 3, 4 and 5 of the document entitled Classification of Etiologic Agents on the Basis of Hazard, as published by the Center for Disease Control (CDC), United States Public Health Service or from oncogenic viruses classified by the National Cancer Institute as moderate risk. The CDC document categorizes naturally occurring organisms and viruses known to be pathogenic to man and agriculturally important species on a scale of increasing hazard, going from 1 to 5. Class 5 agents are excluded from the U.S. by law. Class 4 includes such agents as wild type smallpox virus, wild type yellow fever virus, *Herpesvirus simiae*, and Lassa virus. Class 3 includes *Brucella*, arboviruses and agents causing encephalitis, psittacosis agents, *Rickettsiae* and vesicular stomatitis virus. Class 2 includes agents which may produce diseases of varying degrees of severity if accidentally inoculated into laboratory workers, but which are considered normally containable by standard laboratory practices. Examples of class 2 agents are various species of *Salmonella*, agents causing amoebic dysentery, and mumps, measles and rubella viruses. Class 2 agents may be used in recombinant DNA experiments. The NCI classifies agents such as feline sarcoma and leukemia viruses, and woolly monkey fibrosarcoma virus as moderate risk.

(*2*) Deliberate formation of recombinants containing the genes for toxins of very high toxicity. Examples of this class are botulinus toxin or diphtheria toxin, and venoms from insects and snakes.

(*3*) Deliberate creation from plant pathogens of recombinant DNAs that are likely to increase either the virulence of the pathogenic material or the range of species susceptible to the disease.

(*4*) Certain of the possible beneficial applications of DNA recombinant research involve the creation of organisms with the ability to carry out useful environmental functions. Release of such organisms into the environment may at some point be required to test their efficacy, and certainly to make

use of them. The guidelines state that deliberate release of any organisms containing a recombinant DNA molecule is not to be undertaken at present.

(*5*) Transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

(*6*) Experiments must be limited in scale to quantities of fluid less than 10 l with recombinant DNAs known to make harmful products. The guidelines state that the Advisory Committee may make exceptions to this rule for particular experiments deemed to be of direct societal benefit if appropriate equipment is used.

The use of bacterial hosts and vectors. Recognizing the relation between the host-vector system required by the experiment and the design of suitable biological containment, experiments using the same host-vector system are grouped together. At present, the system of choice for many experiments is the common laboratory bacterium, *E. coli* strain K12, and independent genetic elements (plasmids and bacteriophage) known to reside or replicate in this strain. There are several factors contributing to this conclusion. Strain K12 has been studied extensively and can be readily manipulated for recombinant DNA experiments. This same extensive experience and ease of manipulation permits modification of *E. coli* K12 and the vectors by classical genetic techniques, for the purpose of establishing biological containment. The guidelines also discuss arguments against the use of *E. coli* K12, in particular the intimate association of various other strains of *E. coli* with humans. For this reason the guidelines urge that efforts be made to develop alternate bacterial host-vector systems. For this reason also, the guidelines recommend the cautious use of *E. coli* K12 host-vector systems.

The nature and manner of achieving biological containment with this system is described in the guidelines. *E. coli* K12 appears to be harmless itself, it does not usually either establish itself in the normal bowel, or multiply significantly in the alimentary tract. These facts suggest that accidental ingestion of a small number of bacteria by a laboratory worker would not result in extensive spread of the bacterium outside the laboratory. The normal situation may be altered when people are either taking antibiotics, or have certain abnormal digestive conditions and it is recommended that such individuals refrain from work for the duration of the abnormal situation. However, while *E. coli* K12 does not establish itself as a growing strain in normal bowels, it does remain alive during its passage through the tract. Therefore transfer of plasmid or bacteriophage vectors containing foreign DNA from the original *E. coli* K12 host to bacteria resident in the intestines or bacteria encountered after excretion must be considered. The guidelines specify the use of nonconjugative plasmids as vectors in recombinant research, because they cannot promote their own transfer. However transfer of a resident nonconjugative plasmid is possible in nature if the recombinant-containing host acquires a conjugative plasmid that is derepressed for transfer. For any given host-plasmid combination used in a recombinant DNA experiment it will be necessary to assess the

possibilities for transfer of the recombinant DNA in order to evaluate the degree of biological containment. While we are missing some relevant information, the available data suggest that the probability of transfer can be quite low, depending on the particular nonconjugative plasmid used, on whether or not the conjugative plasmid is repressed with respect to expression of donor fertility, and on the viability of the host cell in natural environments. With certain known and useful plasmids, triparental matings involving first the acquisition of a conjugative plasmid and second, transfer of the nonconjugative plasmid to a third cell occur at frequencies that are less than one in 10^9 and in fact are usually undetectable under laboratory conditions designed to resemble natural conditions. Host-vector systems made up of *E. coli* K12 and such plasmids therefore appear to have only very limited ability to spread recombinant DNA molecules.

Analogous considerations apply when bacteriophage are to be used as vectors for foreign DNA. Bacteriophage vectors could be spread either as mature phage, or in cells either lysogenic for the phage or carrying the phage as a plasmid. The bacteriophage lambda can be used to illustrate relevant considerations since this widely studied bacteriophage is most likely to be used for recombinant experiments at the present time.

Considering first escape as a phage particle, lambda is sensitive to the acidity of the stomach and is likely to be destroyed there. Normal intestinal strains of *E. coli* are usually not susceptible to infection by lambda and in fact, susceptible strains are rare in nature. Further, in at least one case, ingestion of 10^{11} lambda particles yielded no detectable lambda in resulting feces. Lambda is also readily destroyed by drying in air. Dissemination of lambda recombinants through lysogen formation, a frequent event with susceptible *E. coli* strains, can be minimized by use of mutant varieties of lamba which lack genes necessary for lysogen formation: with such phage the frequency of integration into the host chromosome is reduced to 10^{-5} or 10^{-6}. Finally, conversion of lambda DNA to a stable plasmid is also a relatively unlikely event, occurring at a frequency of about 10^{-6}.

Considering then the properties of *E. coli* K12, as well as those of the existing plasmid and bacteriophage vectors, the proposed guidelines conclude that, using such host-vector systems, recombinant DNAs are unlikely to be spread by the ingestion or dissemination of the few hundred or thousand bacteria, such as might be involved in laboratory accidents, given standard microbiological practice. Therefore, these existing systems, and analogous combinations of *E. coli* K12 with other vectors and bacteriophages are judged to offer a moderate level of biological containment and are defined as EK1, the lowest level of biological containment for experiments with *E. coli* systems. Other prokaryote host-vector systems need to be evaluated using the same general principles as those applied to the *E. coli* K12 situation.

As with physical containment levels, increasing numbers specify increasing levels of biological containment for *E. coli* systems. The next level is called EK2. EK2 host-vector combinations must be demonstrated to provide a high

level of biological containment by suitable laboratory tests. They are obtained by genetic modification of either *E. coli* K12 host cells or the relevant plasmids and bacteriophage or both. More specifically, the guidelines state that in order to qualify as EK2 the modified system composed of derivatives of *E. coli* K12 combined with a particular vector should not permit survival of a genetic marker carried on the vector in other than specially designed laboratory environments at a frequency greater than 10^{-8}. Various examples of the types of necessary modifications are suggested in the guidelines. For example, modifications of the host might be mutations which result in special nutritional requirements for growth or sensitivity to naturally occurring materials such as bile salts, or elimination of host-controlled restriction and modification. Suggested modifications of plasmid vectors include mutations making essential plasmid functions sensitive to normal body temperatures or dependent on a specific host. Mutations which make native phage particles containing a recombinant DNA unstable in natural environments and therefore unlikely to infect new *E. coli* cells should they escape can be considered.

One additional level of contained *E. coli* host-vector systems is defined in the guidelines and is called EK3. EK3 systems are EK2 systems for which the specified containment properties have been demonstrated not only by microbiological and genetic analysis but by appropriate tests in animals including humans or primates and other relevant environments.

EK2 and EK3 host vector systems must be certified as such by the Director of NIH, after evaluation and recommendation by the Advisory Committee. Detailed data on the relevant properties of the system must be submitted for consideration by the Committee. Thus far (January, 1977) the following EK2 systems have been certified: *E. coli* strain χ1776 with either plasmid pSC101 or plasmid pCR1, a derivative of colE1, and one lambda phage derivative [3]. Several other phage lambda derivatives, to be used in conjunction with specified partially disarmed *E. coli* K12 host cells, are under consideration by the Committee. No EK3 systems have been submitted for certification as yet. Information concerning certified systems and their availability can be obtained from the office mentioned in ref.1 [4].

Classification of experiments currently permissible with E. coli *K12 host-vector systems*

Having defined the several levels of physical containment and biological containment the specific recommendations for experiments using the *E. coli* K12 host-vector systems can be described. Each type of experiment is assigned both a physical containment level, that is a P level, and a biological containment level, that is an EK level and the particular combination of the two reflects the severity of the estimated potential hazard. The Guidelines are organized, for the *E. coli* systems, according to the source and nature of the foreign DNA, as outlined in Fig.2. A sample of DNA containing essentially

Experiments with *E. coli* "Host-Vector" Systems
Nature of the 'Foreign' DNA

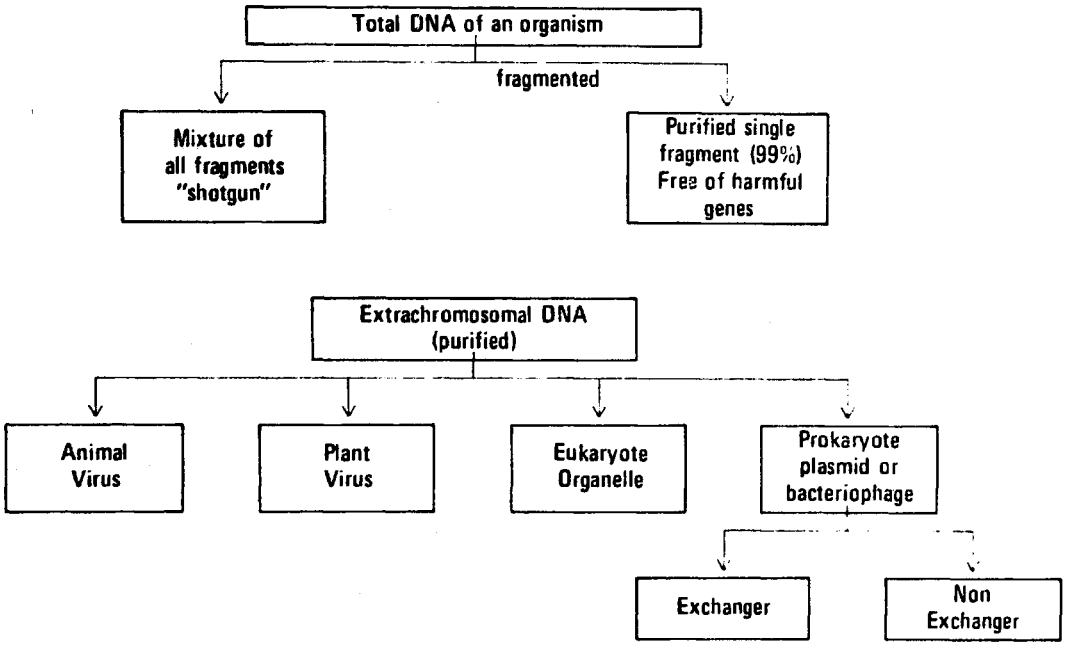

Fig.2. Source and nature of the foreign DNA.

all the genetic information of an organism can be isolated and fragmented. If the experiment involves such a mixture of DNA fragments, it is referred to as a "shotgun" and will call for a certain level of containment. Experiments involving such mixtures of DNA fragments are assumed to be of relatively high potential hazard because of the greater likelihood of unknown and therefore maybe hazardous genes being introduced into a recipient cell compared to experiments with a single, highly purified fragment. Purified fragments containing mainly genes whose properties are known and are not harmful, offer less potential hazard than a shotgun experiment. In some instances the foreign DNA will itself be derived from extrachromosomal genetic elements. Such extrachromosomal elements include the DNA of animal viruses, plant viruses, other eukaryote organelles such as mitochondria and chloroplasts, as well as prokaryote plasmids or bacteriophages, of the same type used as vectors. Each of these cases is treated separately in the guidelines. The prokaryote sources are treated differently, depending on whether the source of the "foreign" DNA is an organism that does or does not exchange genetic information with *E. coli* in nature.

Guidelines for experiments with E. coli *host-vector systems.* Table I shows the containment required for shotgun experiments when the foreign DNA is a mixture of fragments derived from eukaryotes. The physical and biological containment is listed for various possible DNA sources: both must be used as they complement each other. For example, DNA from primates requires the most stringent containment, since the estimated potential hazard either from

TABLE I

Guidelines for experiments with *E. coli* host-vector systems using foreign DNA derived from eukaryotes. "Shotgun" experiments

Foreign DNA source	Containment		
	Physical		Biological
Primates	P3	or	EK3
	P4		EK2
Embryonic	P3		EK2
Other mammals	P3		EK2
Birds	P3		EK2
Cold-blooded vertebrates	P2		EK2
Embryonic	P2		EK1
Toxin	P3		EK2
Lower eukaryotes	P2		EK1
If pathogenic or toxigenic	P3		EK2
Plants	P2		EK1
If pathogenic or toxigenic	P3		EK2

genes that might function in humans with untoward effects, or from pathogenic viral DNAs residing in primate tissue is judged to be most serious. The experiments require either P3 and EK3, or P4 and EK2, and it should be recalled that only the latter combination, P4 and EK2, is feasible at present and even then only at the limited number of P4 facilities. Another point of interest is that in two instances, primates and cold blooded vertebrates, containment requirements are lower if the DNA is isolated from embryonic tissue, or germ line material, since such material is less likely to be contaminated by pathogenic viruses or other adventitious agents than is adult tissue. Thus, if the foreign DNA is from cold-blooded vertebrates, P2 and EK2 are required but P2 and EK1 can be used if the DNA is from embryonic or germ line tissues. If the cold blooded vertebrate is known to produce a potent toxin, P3 and EK2 must be used. In some instances, lower eukaryotes for example, the guidelines require more or less stringent conditions depending on whether or not the source of foreign DNA is known to be pathogenic or toxigenic, or might be infected with a pathogen, or is known to make a harmful product.

Table II summarizes the guidelines for shotgun experiments when the source of the DNA is a prokaryotic organism. First, those prokaryotes which are known to exchange genetic information with *E. coli* in nature are considered. The containment requirements are low for this group and vary with the pathogenicity of the source of foreign DNA. When the source of foreign DNA is a prokaryote that does not naturally exchange genetic material with *E. coli*, the containment recommendations are high. It is assumed that the more similar the DNAs of donor and host, the greater the probability of expression of foreign DNA, or of possible derepression of host genes. In those cases where the donor exchanges DNA with *E. coli* in nature, it is unlikely

TABLE II

Guidelines for experiments with *E. coli* host-vector systems using DNA derived from prokaryotes. "Shotgun" experiments

Foreign DNA source	Containment		
	Physical		Biological
Prokaryotes:			
"Exchangers"			
Class 1 (CDC)[a]	P1		EK1
Class 2 (CDC)	P2		EK1-EK2[b]
Plant pathogens	P2		EK1-EK2[b]
"Non-exchangers"			
Class 1 (CDC)	P2	or	EK2
	P3		EK1
Class 2 (CDC)	P3		EK2
Plant pathogen	P3		EK2

[a] CDC refers to the classification of Etiologic Agents on the Basis of Hazard, as Published by the Center for Disease Control (CDC), United States Public Health Service.

[b] EK1 or EK2, depending on whether the species is of low or moderate pathogenicity.

that recombination experiments will create new genetic combinations, never tested by nature. When prokaryote donors not known to exchange DNA with *E. coli* in nature are used, however, there is a greater potential for new genetic combinations to be formed and expressed.

Characterized clones obtained from shotgun experiments may not be as potentially hazardous as the original mixture of cells. Cloning of the recipient host cell containing the DNA fragment of interest will be one of the normal aims of any recombinant experiment. The guidelines state that when a clone has been obtained from a shotgun experiment, and has been rigorously characterized, and when there is sufficient evidence that it is free of harmful genes, then experiments involving the clone can be carried out under P1 and EK1 conditions if the foreign DNA was from a species that exchanges genes with *E. coli* in nature, and under P2 and EK1 conditions if it does not.

Similarly, when the initial recombination involves a purified segment of the foreign chromosomal DNA, rather than a mixture, the potential for growth of a hazardous organism will be less, since the number of clones that must be examined to obtain the desired clone is markedly reduced. The guidelines define purified (or enriched) as meaning that the desired segment represents at least 99% of the total DNA in the preparation, by weight, and further, they require evidence that no harmful genes are present. Under such circumstances the investigator may lower the containment conditions from these recommended for shotgun experiments with DNA of the same source, either by one step in physical containment or one step in biological contain-

ment. Thus, for example, shotgun experiments with DNA from birds require P3 and EK2. A DNA fragment from birds that is free from harmful genes, and purified to 99% purity prior to joining to a vector, would require either P2 and EK2 or P3 and EK1.

The final group of experiments utilizing *E. coli* host-vector systems that are considered are those in which the foreign DNA is itself from an extrachromosomal element. As indicated in Fig.2 it is assumed that such DNA is purified away from chromosomal DNA prior to recombination. Various possible sources of extrachromosomal DNA are listed in Table III and the recommended combined containment given. For example, DNA from all or part of the genome of an animal virus requires P4 physical containment and an EK2 host vector system, or, alternatively, P3 and EK3. When the recombinants have been purified by cloning, and shown to be free of harmful regions of the viral genome, then experiments can be moved to P3 and EK2.

TABLE III

Guidelines for experiments with *E. coli* host-vector systems in which the foreign DNA is from an extrachromosomal element

"Foreign" DNA source	Containment		
	Physical		Biological
Animal viruses	P4	or	EK2
	P3		EK3
If cloned, free of harmful genes	P3		EK2
Plant viruses	P3	or	EK1
	P2		EK2
Eukaryote organelles[a]	P3	or	EK1
Primates	P2		EK2
Other	P2		EK1
Prokaryote (plasmids and phage)			
"Exchangers"			
Non-pathogens	P1		EK1
Pathogen	As for shotgun		
"Non-exchangers"	As for shotgun		

[a] The organelle DNA must be purified from isolated organelles. Otherwise conditions indicated for shotgun experiments apply.

When complementary DNAs (cDNA), synthesized in vitro from RNA preparations, are used in recombination experiments, the containment requirements are as described for isolated DNA preparations. Thus, for example, if the cDNA is less than 99 per cent pure, shotgun conditions are required.

Guidelines for experiments with animal host-vector systems. Many recombinant DNA experiments will involve the use of systems in which the host cells are eukaryote cells grown as single cells in tissue culture: useful vectors may include extrachromosomal DNA elements such as organelle DNA, or the DNA of viruses that infect the particular cells of interest. Given the current state of technology, viral DNAs are most likely to be used as vectors in the near future. The cells themselves are fragile and fastidious and there is little or no chance that a living cell could escape from a laboratory in the way that an *E. coli* cell might. Therefore containment considerations focus on the viruses. Animal viruses can escape a laboratory in a viable form, especially if laboratory workers become infected. There are two animal viruses whose DNAs are, now, technically useful as vectors; polyoma and simian virus 40 (SV40). The cleavage of these molecules with restriction endonucleases has been studied entensively. In their respective normal hosts, mouse for polyoma, rhesus monkeys for SV40, neither virus causes a known disease. Polyoma does not infect human cells grown as single cells in the lab and also does not appear to infect humans, since humans exposed to polyoma do not produce antibodies. SV40 does infect both human cells grown as single cells in the laboratory, and whole human beings, as evidenced by the active production of antibodies and the reports of isolation of SV40 from humans. This virus contaminated the early Salk polio vaccines and millions of people were inadvertently inoculated with it in the middle 1950s. To date, there is no indication that the recipients of the vaccine suffered any related difficulty. Both polyoma and SV40 are oncogenic, that is they cause tumor formation in newborn small laboratory mammals, and both can transform a variety of cells of mammalian origin. They are classified as low risk oncogenic viruses by the National Cancer Institute, and the viruses themselves must be handled under conditions equivalent to P2. Because SV40 infects human beings, and also because SV40 and related viruses have been isolated in connection with several human disease states, the proposed guidelines assume that polyoma inherently affords a higher level of biological containment: therefore more stringent physical containment is required for SV40 than for polyoma.

The guidelines require that the viral DNA used for recombination with a foreign DNA must itself be defective, that is, its propagation as a virus must be dependent on the presence of helper virus which supplies the genes for the missing functions. This helper can be another defective or an appropriate conditional lethal mutant virus. Alternatively the helper might be previously integrated into the genome of a stable line of host cells. The use of a nondefective genome as a helper is permissible if the alternatives are unavailable. In certain kinds of experiments, no production of viral particles is required, and no helper may be needed: biological containment is inherently greater in the absence of virus particles since, as pointed out before, cells themselves are relatively easy to contain.

With these aspects of biological containment in mind, the guidelines specify required physical containment for these experiments, as summarized in

Table IV. The particular levels of physical containment depend on the source of the foreign DNA, whether defective polyoma or defective SV40 is the chosen vector, and finally on whether or not virus particles are produced.

Considering first experiments with defective polyoma vectors, under con-

TABLE IV

Guidelines for use of polyoma and SV40 as vectors

"Foreign" DNA	Physical containment	
	Polyoma	SV40
With virion production		
Non-pathogen	P3	P4
If purified, cloned, harmless	P3	P3
Low pathogenicitv	P4	—
No virion production		
Non-pathogen	P3	P3
Low pathogenicity	P3	—

ditions where viral particles are produced... if the foreign DNA is from a nonpathogenic agent, P3 conditions are required, even if the DNA fragment was purified first and does not contain harmful genes. If the foreign DNA is from an organism with low pathogenicity, P4 must be used until such time as suitable tests indicate that only harmless genes are present and then experiments can be continued at the P3 level. Still considering polyoma vectors, P3 conditions are required for experiments in which no virus particles are produced. When defective SV40 is the vector, and virus particles are produced, P4 conditions must be used and the foreign DNA must be from a nonpathogenic organism. Experiments can be done in P3 only after extensive and specified kinds of purification of the DNA and demonstration that no genes for toxic products are present. SV40 can not be used at all for experiments with DNA from pathogenic organisms. When no SV40 virus particles are produced, experiments with recombinants derived from nonpathogenic agents can be carried out in P3 conditions.

Guidelines for experiments with plant host vector systems. The Guidelines also contain recommendations for experiments in which plant cells will serve as hosts for recombinant DNA. The cells might be single plant cells grown under laboratory conditions, or seedlings, plant parts, or small whole plants. This is in fact the only instance where the guidelines address the question of recombinant DNA experiments with whole organisms. Directions are given for modification of the specifications for P1, P2, and P3 physical containment in order to provide conditions appropriate for work with plants.

Vectors for use in experiments with plants include plant organelle DNA

such as the DNA of chloroplasts, and DNA of viruses of low pathogenicity and restricted host range. These vectors offer moderate levels of biological containment, and the guidelines specify the physical containment levels outlined in Table V. As before, the requirements are organized according to the

TABLE V

Experiments with plant host-vector systems

Source of "foreign" DNA	Physical containment
Species in which vector can replicate	P2
If harmful products possible	P3
Other species	
Foreign DNA purified (99%), and no harmful genes	P2
Foreign DNA not purified, and no harmful genes	P3
Foreign DNA contains harmful genes	P4

source of the foreign DNA. If the foreign DNA is derived from a species in which the vector DNA is known to be able to replicate, P2 conditions are required, unless the source of foreign DNA is pathogenic or produces products dangerous to plants... then P3 is required. If the foreign DNA is derived from a species in which the vector is not known to replicate then more stringent requirements govern and vary from P2 to P4 depending on whether the DNA is purified, and whether it contains harmful genes.

Other host-vector systems. Theoretically, there are a variety of organisms, both prokaryotes and lower eukaryotes such as fungi and yeast which will be interesting and useful hosts for experiments with recombinant DNAs. Some may offer the special advantage of not infecting humans, animals or important ecological niches. However, a variety of technical developments are needed before useful vectors are available for these systems. The growth characteristics of such hosts indicate that containment problems will be like those for *E. coli* K12 hosts. The guidelines urge development of these systems and point out that the detailed recommendations made for *E. coli* K12 systems can be used as a guide in determining biological and physical containment requirements for these systems when that is required.

Implementation of the Guidelines. The guidelines contain a large section defining the roles and responsibilities of individuals and institutions in assuring compliance with required containment levels. The procedures, as described, are primarily directed at grantees of the National Institutes of Health. Similar procedures are in force for work carried out within the NIH laboratories themselves, and for work carried out under contract arrangements with the NIH.

The principal investigator is required to assess any potential biohazards, to institute appropriate safeguards and procedures, to minimize effects of possible accidents by planning, to train and inform all personnel, to report any serious or extended illness of a worker or any accidents, and all of these must be carried out on a continuing basis. Thus, the primary responsibility for conducting experiments according to the guidelines is in his hands. Further, in applying for grants to carry out experiments with recombinant DNA, the investigator must include an estimate of the potential biohazards as well as a statement as to the containment procedures that will be used. The application must include certification as to the existence and availability of appropriate facilities, procedures, and training. The guidelines indicate that institutions in which recombinant DNA experiments are carried out must establish biohazard committees which can serve to examine equipment and facilities and certify their compliance with the requirements. Such committees will also serve as a source of advice and reference on physical containment facilities, on properties of biological containment, and on training of personnel.

According to the proposed guidelines review of the certification and of the investigator's judgment concerning the extent of potential hazard and the required containment would be by NIH study sections, during the normal scientific review of the application. The guidelines leave flexible the question of resolving any differences between the evaluation of the investigator and that of the study section. The guidelines do state, however, that in instances where resolution of differences cannot be made, the matter should be referred to the Advisory Committee or the NIH Office of Recombinant DNA Activities.

Application of the guidelines to work not supported by the National Institutes of Health. Several agencies of the U.S. government other than the National Institutes of Health provide support for biological and medical research. Some of these agencies are currently, or may in the future, sponsor recombinant DNA experiments. Adoption of the NIH guidelines is being considered by these agencies. At this writing (January, 1977) the following have stated that the Guidelines will be applicable to their grantees: the National Science Foundation, the Energy Research and Development Administration, the National Aeronautics and Space Administration, and the Department of Defense.

Efforts are also underway to develop appropriate and effective ways to extend the requirements in the guidelines to research supported by private funds. In the meanwhile it is anticipated that voluntary compliance by the private sector will be extensive.

Guidelines in other countries. The United Kingdom [5] has independently developed guidelines for research with recombinant DNA. Comparison of the American and British guidelines suggests that they are similar, although precise comparison is difficult because of different definitions of physical containment categories and because of differing approaches to implementation, required by differing national procedures. The British document has somewhat more stringent definitions than the NIH guidelines for the lower levels

of physical containment (P1 through P3): the NIH P4 specifications appear to be more stringent than the comparable level in the British document. No experiments are specifically prohibited by the British Report.

More precise comparison will be possible as specific experiences accumulate[6].

1 Copies of this document may be obtained from the Executive Secretary, Office of Recombinant DNA Activities, Building 31, National Institutes of Health, Bethesda, Maryland, 20014, (U.S.A.).

2 Berg, P., Baltimore, D., Brenner, S. and Singer, M.F., Science 188 (1975) 991; Nature, 225 (1975) 442; Proc. Natl. Acad. Sci. USA, 72 (1975) 1981.

3 Enquist, L., Tiemeier, D., Leder, P., Weisberg, R., Sternberg, N., Nature, 259 (1976) 596–598.

4 Scientists involved in recombinant DNA research may keep informed of further developments concerning host-vector systems as well as other aspects of the field by subscribing to the "Nucleic Acid Recombinant Scientific Memoranda". Inquiries should be addressed to: Dr. E.C. Chamberlayne, Project Officer, FIC. Building 31, Room 2C15, National Institutes of Health, Bethesda, Maryland, 20014 (U.S.A.).

5 Report of the Working Party on the Practice of Genetic Manipulation, June, 1976 London, Her Majesty's Stationery Office.

6 A detailed review of activities in many countries has been prepared by John Tooze, European Molecular Biology Organization, 69, Heidelberg, Germany.

Communicated by W. Szybalski.